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SHAUGHNESSY NO.

REVIEW NO. _____

EEB REVIEW

DATE: IN 8/9/89

DATE: OUT _____

FILE OR REG. NO. 59011

PETITION OR EXP. NO. _____

DATE OF SUBMISSION 7/28/89

DATE RECEIVED BY EFED 8/9/89

RD REQUESTED COMPLETION DATE 9/1/89

EEB ESTIMATED COMPLETION DATE 9/1/89

RD ACTION CODE 660

TYPE OF PRODUCT(S) : I,D,H,F,N,R,S Herbicide

DATA ACCESSION NO(S). _____

PRODUCT MANAGER (NO.) C. Grubbs (74)

PRODUCT NAME(S) Trifluralin

COMPANY NAME Elanco Products Co.

SUBMISSION PURPOSE Submission of aquatic laboratory study protocol
designed to address vertebral lesions in freshwater fish
species (and to replace field testing requirement 72-7)

SHAUGHNESSY NO.

CHEMICAL & FORMULATION(S)

% A.I.

EEB REVIEW

100 Chemical: Trifluralin

102 Purpose of Submission

The purpose of this study is to conduct a laboratory study to establish a chronic no-effect concentration for vertebral lesions.

103 Background Information

In previous correspondence, Elanco had provided data that presumably demonstrated a no-effect level for vertebral dysplasia in fish exposed in the laboratory, that concentrations of trifluralin in surface water were below vertebral effect concentrations and vertebral lesions identified in the field were not considered as trifluralin related. EEB did not agree.

In a subsequent letter from Akerman (EEB) to Elanco (2/7/89), the registrant was required to:

- 1) conduct a field monitoring study to principally monitor body burden residues in finfish. If residues occur at levels of concern, ≥ 10 ppb whole body, then
- 2) a laboratory study to establish a chronic no-effect concentration for vertebral lesions and/or extensive finfish population monitoring would be warranted.

In a meeting between Elanco and EEB on 7/27/89, Elanco proposed to conduct a laboratory study on finfish to establish a no-effect level for vertebral lesions without first conducting the field study. Several options for the laboratory study were discussed; e.g., using the early life-stage or exposing larvae or older fish to trifluralin. Elanco then agreed to submit a proposed test protocol based on these discussions. The proposed protocol is attached.

103 Proposed Study

The basic study design follows the fish early life stage, flow-through, test design.

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Test concentrations will be maintained during the test by use of a proportional flow-through diluter system. Beginning with a nominal stock of 42.5 $\mu\text{g/L}$. Every 12 min an aliquot will be delivered to an appropriate volume of conditioned well water to create a high test concentration of 8 $\mu\text{g/L}$; this will be proportionately diluted to provide the following test concentrations: 0.5, 1.0, 2.0, 4.0 and 8.0 $\mu\text{g/L}$. Each aquarium will receive approximately 11 volume replacements/day.

Test concentrations were based on literature information. The life-cycle MATC for fathead minnows was between 1.9 and 5.1 $\mu\text{g/L}$. An early life stage study with sheepshead minnows indicated that 5.5 $\mu\text{g/L}$ caused vertebral dysplasia.

To verify test concentrations, it is proposed that for each test concentration, a composite sample will be collected from the 4 replicates at each test concentration be collected on approximately a weekly basis. Approximately 35 composite samples total (5 times x 7 concentrations). One composite sample for each concentration will be collected at the beginning and termination of the experiment; also one composite will be collected each test concentration on a weekly basis. Samples will be handed to a laboratory for analyses.

Eggs will be collected from Lilly Environmental Toxicology Laboratory Stock. The test will begin with eggs less than 24 hrs old; the test will run for 33 days (28 days post-hatch). A total of 120 fish will be exposed to each of 5 test concentrations, an acetone control and a water control. For each test concentration and control, 30 embryos will be exposed per cup, and 4 cups will be exposed per test concentration. After hatching, larvae will be transferred to 2.5 gallon aquaria for the duration of the study.

Test conditions include use of conditioned well water (presumably the same water that the fathead minnows are cultured in), $25 \pm 2^\circ\text{C}$, 16L:8D photoperiod, 400-800 lux light intensity, dissolved oxygen (DO) concentration > 60% saturation, and ammonia < 0.020 mg/L. Fish will be fed Artemia nauplii 3 times on week days and 2 times on weekends during the study. Fish will be fasted 24 hrs before test termination.

Basic physical and chemical parameters, e.g., DO, pH and temperature will be monitored daily in one replicate of each test concentration. Total hardness, total alkalinity, conductivity and ammonia will be measured

weekly in one replicate of the acetone control and the highest test concentration.

Parameters to be measured on eggs include daily observations of non-viable eggs for viability/non-viability (mortality), time to hatch and percent hatch.

Parameters to be measured on the larvae include observations on development, behavior and survival every Monday, Wednesday and Friday. Dead fish will be removed.

At the end of each test, the surviving fish will be photographed and total length will be determined for each surviving fish. The wet weight for each surviving fish will be obtained. A minimum of 10 fish from each treatment will be examined histologically for vertebral injury. Tissue samples will also be submitted for analysis of trifluralin residues.

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EEB Comments on Study

In the protocol cover letter (D. Lade, Elanco), states that "the Agency agreed with Elanco that the Actual Field Testing study should be replaced by a laboratory test designed to identify the no-effect level for vertebral lesions in fresh water fish species." The Agency does not agree that the laboratory test could replace the field study. Rather, since a possible result of the field study identified in the EEB letter (dated February 7, 1989) would be to conduct a laboratory study to establish no-effect level for vertebral lesions, it was not unreasonable for Elanco to proceed directly to a laboratory study.

Depending upon the results of the laboratory study, a field study may still be necessary to support the continued registration of trifluralin. A continuing issue of concern is the need for adequate documentation that surface water concentrations of trifluralin are well below the vertebral effect concentrations.

A laboratory study is appropriate to emphasize the effects of trifluralin and diminish the impact of other factors that cause vertebral and spinal abnormalities; e.g., parasitic infection, vitamin deficiencies, altered hormone balance (presumably due to a variety of environmental factors), radiation, salinity, dissolved oxygen, temperature, radiation, electric current (AC), trauma, various anthropogenic chemicals and naturally occurring trace heavy metals.

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The basic concept of using an early life stage is reasonable. The use of fathead minnows (1.9 ppb) may be acceptable since the life cycle NOEL is similar to that for sheepshead minnow (1.3 ppb) and Daphnia magna (2.4 ppb). A continuous chronic exposure maybe preferred to a pulse-dose exposure to determine the impact of a more "long-term" exposure on developing fish; however, a single "pulse" of trifluralin in a stream caused permanent vertebral dysplasia in trout (Wells and Cowan 1982).

The following questions must be answered and modifications made to the protocol before it is acceptable.

- 1) The selection of a fathead minnow as a test organism seems reasonable. However, young catfish may be a better test organism because the bones ossify faster, they are more sensitive to chemical effects on bone, and they would be better representative of a warm water fish (Dr. P. Mehrle, pers. comm. on 8/2/89, FWS, Columbia MO).
- 2) A 28 day exposure, following the typical early life stage protocol, may not be sufficient to generate the information desired from this test. There will not be sufficient time for the backbone of the fathead minnow to ossify sufficiently to determine if there is an effect or not. Elanco must consider one of these options (Mehrle, pers. comm.):
 - a) beginning with a typical early life stage test and continue exposure for 56 days post-hatch. Couch et al. (1979) found that vertebral dysplasia was more pronounced in sheepshead minnow exposed for more than 50 days (zygote to adults); or
 - b) starting the test with 30 day old fathead minnows or 21 day old catfish and expose them for a minimum of 28 days to trifluralin.

Elanco must also provide a rationale for not maintaining the fish for an additional 30 days post-exposure. Depuration rates for trifluralin range from 17 to 40 days. High body residues may provide a continued effect on the vertebrae following exposure. If a post-exposure period is included in the design, then provisions must be made to measure fish body and tissue burdens at the termination of exposure and termination of the experiment, i.e., at end of post exposure period.

- 3) The type of measurements that will be used to identify vertebral anomalies must be specified in the protocol. At a minimum, it is assumed that Elanco will use the same measurements utilized by Couch et al. (1979) which included thickness of the vertebral wall (hyperostosis), dorsal outgrowth of the vertebrae (resulting in compression of the spinal cord), ventral outgrowth of the vertebrae (with compression of the mesonephric ducts) and fusion of the vertebrae. Other measures, such as the Shape Factor used by Wells and Cowan (1982), may also be appropriate. The measurements that will be made must include those obtained for the fish in a previous field study since it was those results which continue to be of concern to the EEB.

Elanco contends that the histological measurements on developing larvae are more sensitive than other types of measurements made on older fish. A comparison of the histological method with other methods and measurements must be presented so that EEB is convinced that the type of measurements to be used by Elanco are sensitive and replicable.

While more sensitive parameters can be measured, e.g., ratio of calcium and phosphate to collagen, and backbone elasticity/tensile strength their utility in this study may not be appropriate (Mehrlle, pers. comm.).

- 4) The highest test concentration may not be high enough to generate mortality, growth impairment or vertebral anomalies. Higher concentrations must be included to obtain significant differences from background effects (which may range from 2 to 5%), especially vertebral anomalies due to heredity, defective embryonic development, poor nutrition and trauma (laboratory setting).

It is also not clear if the lowest test concentration is sufficient to generate a no-effect concentration (or NOEL). If the study does not generate a NOEL, the study will need to be repeated at lower test concentrations.

- 5) Verification of trifluralin concentrations during the test is extremely important to determine the validity of the study and interpret the results. Weekly measurements, based on composite samples from replicates within each concentration, are not sufficient to guarantee that the fish were exposed to the proposed test concentrations. More frequent
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measurements, and measurements between replicates (not composited samples), will be needed to accurately calculate the mean test concentrations and the range of exposures within a test concentration and among test concentrations for each time period.

Temporal variability in chemical concentrations is expected in flow-through tests. For example, changes in water pressure will change flow rates and result in fluctuations in the chemical concentrations that the fish will be exposed to during the test. Further, because the differences between test concentrations is small, it may be very difficult to prevent overlap between effective chemical concentrations. Therefore, it may be difficult to interpret the test results and satisfy this data requirement.

- 6) Elanco must provide information that their analytical techniques for trifluralin are sufficient to determine trifluralin residues in water, and fish whole body and tissues. The amount of biomass needed to accurately detect and measure body residues is important because it will affect the number of fish that will need to be exposed (see below).
- 7) A minimum of 120 fish will be required to be exposed to each test concentration and control regardless of the test method or fish used. Thirty fish ($\geq 25\%$ of the survivors) randomly selected from each treatment and controls must be examined for vertebral anomalies. EEB does not consider studies on a minimum of 10 of 120 exposed fish per treatment and control (8.3%) to be adequate to resolve one of the major questions being addressed in this study. Fish must be randomly selected from each replicate within each test concentration.
- 8) It is not clear from the protocol why fish length will be measured from photographs and then each fish will be independently weighted. The Agency understands the desire for a photographic record, but will require simultaneous measurement of weight and length on all surviving fish at the end of the study and that these data will be recorded for each fish. For each fish randomly selected for vertebral studies, these data will also be recorded with the appropriate weight/length data for each fish. If body residues are measured for individual fish, these data must also be recorded with the appropriate weight/length data for each fish.

- 9) While a principle concern of this study is to evaluate trifluralin exposure to incidence of vertebral anomalies, the other data obtained from this study is valuable (survival, growth, etc) and must be included in the final report. All copies of raw data sheets must also be included in the final report submitted to the Agency.

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Conclusions

The Agency requires clarification and/or incorporation of the above concerns before the protocol is acceptable. This study does not replace the field study. Depending upon the results of the laboratory study, a field study may still be necessary to support the continued registration of trifluralin.

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ELANCO

July 28, 1989

Document Processing Desk (RS-0179)
Office of Pesticide Programs - H7504C
U. S. Environmental Protection Agency
401 M. Street (SW)
Washington, D.C. 20460

RE: TRIFLURALIN REGISTRATION STANDARD (CONSORTIUM NO. 59011);
PROTOCOL FOR AQUATIC ORGANISMS-ACTUAL FIELD TESTING 72-7

At a July 27, 1989 meeting between representatives from Elanco and EPA, the attached protocol was agreed to in principle to satisfy the above Field Study requirement.

In attendance representing the Agency were Mr. Jim Akerman, Dr. Art Buikema, Mr. Lawrence Schnaubelt, and Ms. Joanne Miller. At this meeting, the Agency agreed with Elanco that the Actual Field Testing study should be replaced by a laboratory test designed to identify the no-effect level for vertebral lesions in fresh water fish species. Based on these discussions, this proposed protocol will be finalized after review and comment by the Agency.

Sincerely,

ELANCO PRODUCTS COMPANY
A DIVISION OF ELI LILLY & COMPANY

Dennis H. Lade

Dennis H. Lade, Ph.D., Project Manager
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DHL:llt

Enclosure

cc: Office of Compliance Monitoring (EN-342)
Mr. Lawrence J. Schnaubelt
Ms. Joanne I. Miller
Mr. James Akerman
Dr. Art Buikema

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Trifluralin Science Reviews

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